

[1-¹⁴C]-isopentenyl diphosphate (1 Ci/mol) 25 nmol
 Allylic diphosphate (geranyl diphosphate) 25 nmol
 Potassium phosphate buffer (pH 5.8) 10 mM
 MgCl₂ 5 mM

Enzyme solution 100 µg

H₂O to make 200 µl

After the reaction is over, 200 µl of saturated NaCl was added to the reaction solution and 1 ml of water-saturated butanol was added thereto, which was then agitated, 10 centrifuged, and separated into two phases. To 800 µl of the butanol layer obtained was added 3 ml of a liquid scintillator and then the radioactivity was measured by the scintillation counter. The result is shown in FIG. 2.

The mutant prenyl diphosphate synthase has exhibited a 15 thermo stability which is equal to that of the native geranylgeranyl diphosphate synthase, and is higher than that of the farnesyl diphosphate synthase derived from *Bacillus stearothermophilus*.

The solvent is evaporated from the remainder of the butanol layer by purging nitrogen gas thereto while heating the layer in order to concentrate to a volume of about 0.5 ml. To the concentrate were added 2 ml of methanol and one 5 ml of potato acid phosphatase solution (2 mg/ml potato acid phosphatase, 0.5 M sodium acetate (pH 4.7)) to effect the dephosphorylation reaction at 37° C. Subsequently the dephosphorylated reaction product was extracted with 3 ml of n-pentane.

This was concentrated by evaporating the solvent by purging nitrogen gas thereto, which was then analyzed by TLC (reverse phase TLC plate: LKC18 (Whatman), development solvent: acetone/water=9/1). The developed dephosphorylated reaction product was analyzed by the Bio Image Analyzer BAS2000 (Fuji Photo Film) to determine the location of radioactivity. The result when geranyl diphosphate was used as the allylic substrate is shown in FIG. 3.

The reaction product of the mutant prenyl diphosphate synthase was shown to be a farnesyl diphosphate.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(iii) NUMBER OF SEQUENCES: 14

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 330 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Sulfolobus acidocaldarius*
- (B) STRAIN: ATCC 33909

(ix) FEATURE:

- (A) NAME/KEY: Asp-rich domain
- (B) LOCATION: 82-86

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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Met Ser Tyr Phe Asp Asn Tyr Phe Asn Glu Ile Val Asn Ser Val Asn
      5                               10                               15
Asp Ile Ile Lys Ser Tyr Ile Ser Gly Asp Val Pro Lys Leu Tyr Glu
      20                               25                               30
Ala Ser Tyr His Leu Phe Thr Ser Gly Gly Lys Arg Leu Arg Pro Leu
      35                               40                               45
Ile Leu Thr Ile Ser Ser Asp Leu Phe Gly Gly Gln Arg Glu Arg Ala
      50                               55                               60
Tyr Tyr Ala Gly Ala Ala Ile Glu Val Leu His Thr Phe Thr Leu Val
      65                               70                               75                               80
His Asp Asp Ile Met Asp Gln Asp Asn Ile Arg Arg Gly Leu Pro Thr
      85                               90                               95
Val His Val Lys Tyr Gly Leu Pro Leu Ala Ile Leu Ala Gly Asp Leu
      100                              105                              110
Leu His Ala Lys Ala Phe Gln Leu Leu Thr Gln Ala Leu Arg Gly Leu
      115                              120                              125
Pro Ser Glu Thr Ile Ile Lys Ala Phe Asp Ile Phe Thr Arg Ser Ile
      130                              135                              140
Ile Ile Ile Ser Glu Gly Gln Ala Val Asp Met Glu Phe Glu Asp Arg

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145	150	155	160
Ile Asp Ile Lys Glu Gln Glu Tyr Leu Asp Met Ile Ser Arg Lys Thr	165	170	175
Ala Ala Leu Phe Ser Ala Ser Ser Ser Ile Gly Ala Leu Ile Ala Gly	180	185	190
Ala Asn Asp Asn Asp Val Arg Leu Met Ser Asp Phe Gly Thr Asn Leu	195	200	205
Gly Ile Ala Phe Gln Ile Val Asp Asp Ile Leu Gly Leu Thr Ala Asp	210	215	220
Glu Lys Glu Leu Gly Lys Pro Val Phe Ser Asp Ile Arg Glu Gly Lys	225	230	235
Lys Thr Ile Leu Val Ile Lys Thr Leu Glu Leu Cys Lys Glu Asp Glu	245	250	255
Lys Lys Ile Val Leu Lys Ala Leu Gly Asn Lys Ser Ala Ser Lys Glu	260	265	270
Glu Leu Met Ser Ser Ala Asp Ile Ile Lys Lys Tyr Ser Leu Asp Tyr	275	280	285
Ala Tyr Asn Leu Ala Glu Lys Tyr Tyr Lys Asn Ala Ile Asp Ser Leu	290	295	300
Asn Gln Val Ser Ser Lys Ser Asp Ile Pro Gly Lys Ala Leu Lys Tyr	305	310	315
Leu Ala Glu Phe Thr Ile Arg Arg Arg Lys	325	330	

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 993 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Sulfolobus acidocaldarius*
- (B) STRAIN: ATCC 33909

(ix) FEATURE:

- (A) NAME/KEY: Asp-rich domain coding
- (B) LOCATION: 246-258

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

ATGAGTTACT TTGACAACTA TTTTAATGAG ATTGTTAATT CTGTAAACGA CATTATTAAG	60
AGCTATATAT CTGGAGATGT TCCTAAACTA TATGAAGCCT CATATCAITT GTTACATCT	120
GGAGGTAAGA GGTAAAGACC ATTAATCTTA ACTATATCAT CAGATTTATT CGGAGGACAG	180
AGAGAAAGAG CTTATTATGC AGGTGCAGCT ATTGAAGTTC TTCATACITT TACGCTTGTG	240
CATGATGATA TTATGGATCA AGATAATATC AGAAGAGGST TACCCACAGT CCACGTGAAA	300
TACGGCTTAC CCTTAGCAAT ATTAGCTGGG GATTTACTAC ATGCAAAGGC TTTTCAGCTC	360
TTAACCCAGG CTCTTAGAGG TTTGCCAAGT GAAACCATAA TTAAGGCITT CGATATTTTC	420
ACTCGTTCAA TAATAATTAT ATCCGAAGGA CAGGCAGTAG ATATGGAATT TGAGGACAGA	480
ATTGATATAA AGGAGCAGGA ATACCTTGAC ATGATCTCAC GTAAGACAGC TGCATTATTC	540
TCGGCATCCT CAAGTATAGG CGCACTTATT GCTGGTGCTA ATGATAATGA TGTAAGACTG	600
ATGTCGTGATT TCGGTACGAA TCTAGGTATT GCATTTTCTA TTGTTGACGA TATCTTAGGT	660
CTAACAGCAG ACGAAAAGGA ACTTGAAAG CCTGTTTTTA GTGATATTAG GGAGGGTAAA	720

AAGACTATAC	TTGTAATAAA	AACACTGGAG	CTTTGTAAAG	AGGACGAGAA	GAAGATTGTC	780
CTAAAGGCGT	TAGGTAATAA	GTGAGCCTCA	AAAGAAGAAT	TAATGAGCTC	AGCAGATATA	840
ATTAAGAAAT	ACTCTITAGA	TTATGCATAC	AATTTAGCAG	AGAAATATTA	TAAAAATGCT	900
ATAGACTCTT	TAAATCAAGT	CTCCTCTAAG	AGTGATATAC	CTGGAAGGCG	TTTAAATAT	960
CTAGCTGAAT	TTACGATAAG	AAGGAGAAAA	TAA			993

(ii) MOLECULE TYPE: cDNA

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:
GTTCTTCATA CTTATTCGCT TATTCATGAT AGTATT 36

(2) INFORMATION FOR SEQ ID NO:8:
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 33
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:
ATTTCATGATG ATCTTCCATC GATGGATCAA GAT 33

(2) INFORMATION FOR SEQ ID NO:9:
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 27
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:
TTTTTCCTTG TGGCTGATGA TATCATG 27

(2) INFORMATION FOR SEQ ID NO:10:
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 27
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:
TTTTTCCTTG TGCTTGATGA TATCATG 27

(2) INFORMATION FOR SEQ ID NO:11:
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 27
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:
TATTTCTTG TGCTTGATGA TATCATG 27

(2) INFORMATION FOR SEQ ID NO:12:
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 27
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:
TATTTCTTG TGGCTGATGA TATCATG 27